

SEQUENCE LISTING

<110> Eck, Jorgen
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<120> Recombinant Fusion Proteins Based on
Ribosome-Inactivating Proteins of the mistletoe Viscum
album

<130> 09282-5

<140> Not Yet Assigned

<141> 1999-07-02

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<151> 1997-01-02

<160> 38

<170> PatentIn Ver. 2.0

<210> 1

<211> 762

<212> DNA

<213> Viscum album

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ctcttgctgc agtctacgat ccccgctctcc gatgcgcaaa gatttgtctt ggtggagctc 180
accaaccagg ggggagactc gatcacggcc gcatcgcacg ttaccaatct gtacgtcgtg 240
gcttaccaag caggcgacca atcctacttt ttgcgcgacg caccacgcgg cgcggaaacg 300
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gagcgatag ccggacatag ggaccagatc cctctcggtg tagaccaact cattcaatcc 420
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ctcattcaga tgatctccga ggccgccaga ttcaatcca tcttatggag ggctcgccaa 540
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cgggttgcta tcccccccg taacttcgtg acgttgacca atgttcgcga cgtgatcgcc 720
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<210> 2

<211> 252

<212> PRT

jc625 U.S. PTO
09/347064
07/02/99

<213> Viscum album

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Met Tyr Glu Arg Ile Arg Leu Arg Val Thr His Gln Thr Thr Gly Glu
1 5 10 15

Glu Tyr Phe Arg Phe Ile Thr Leu Leu Arg Asp Tyr Val Ser Ser Gly
20 25 30

Ser Phe Ser Asn Glu Ile Pro Leu Leu Arg Gln Ser Thr Ile Pro Val
35 40 45

Ser Asp Ala Gln Arg Phe Val Leu Val Glu Leu Thr Asn Gln Gly Gly
50 55 60

Asp Ser Ile Thr Ala Ala Ile Asp Val Thr Asn Leu Tyr Val Val Ala
65 70 75 80

Tyr Gln Ala Gly Asp Gln Ser Tyr Phe Leu Arg Asp Ala Pro Arg Gly
85 90 95

Ala Glu Thr His Leu Phe Thr Gly Thr Thr Arg Ser Ser Leu Pro Phe
100 105 110

Asn Gly Ser Tyr Pro Asp Leu Glu Arg Tyr Ala Gly His Arg Asp Gln
115 120 125

Ile Pro Leu Gly Ile Asp Gln Leu Ile Gln Ser Val Thr Ala Leu Arg
130 135 140

Phe Pro Gly Gly Ser Thr Arg Thr Gln Ala Arg Ser Ile Leu Ile Leu
145 150 155 160

Ile Gln Met Ile Ser Glu Ala Ala Arg Phe Asn Pro Ile Leu Trp Arg
165 170 175

Ala Arg Gln Tyr Ile Asn Ser Gly Ala Ser Phe Leu Pro Asp Val Tyr
180 185 190

Met Leu Glu Leu Glu Thr Ser Trp Gly Gln Gln Ser Thr Gln Val Gln
195 200 205

His Ser Thr Asp Gly Val Phe Asn Asn Pro Ile Arg Leu Ala Ile Pro
210 215 220

Pro Gly Asn Phe Val Thr Leu Thr Asn Val Arg Asp Val Ile Ala Ser
225 230 235 240

Leu Ala Ile Met Leu Phe Val Cys Gly Glu Arg Pro
245 250

<210> 3
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<212> DNA
<213> Viscum album

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cagttgtggc cctccaagtc caacaatgat ccgaatcagt tgtggacgat caaaagggat 180
ggaaccattc gatccaatgg cagctgcttg accacgtatg gctatactgc tggcgtctat 240
gtgatgatct tcgactgtaa tactgctgtg cgggaggcca ctctttggca gatatggggc 300
aatgggacca tcatcaatcc aagatccaat ctggttttgg cagcatcatc tggaatcaaa 360
ggcactacgc ttacggtgca aacactggat tacacgttgg gacagggtcg gcttgccggt 420
aatgataccg cccacgcga ggtgaccata tatgggttca gggacctttg catggaatca 480
aatggaggga gtgtgtgggt ggagacgtgc gtgagtagcc aaaagaacca aagatgggct 540
ttgtacgggg atggttctat acgccccaaa caaaaccaag accaatgcct cacctgtggg 600
agagactccg tttcaacagt aatcaatata gttagctgca gcgctggatc gtctgggcag 660
cgatgggtgt ttaccaatga aggggccatt ttgaatttaa agaatgggtt ggccatggat 720
gtggcgcaag caaatccaaa gctccgcga ataatcatct atcctgccac aggaaaacca 780
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<210> 4
<211> 267
<212> PRT
<213> Viscum album

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Asp Asp Val Thr Cys Ser Ala Ser Glu Pro Thr Val Arg Ile Val Gly
1 5 10 15
Arg Asn Gly Met Cys Val Asp Val Arg Asp Asp Asp Phe Arg Asp Gly
20 25 30
Asn Gln Ile Gln Leu Trp Pro Ser Lys Ser Asn Asn Asp Pro Asn Gln
35 40 45
Leu Trp Thr Ile Lys Arg Asp Gly Thr Ile Arg Ser Asn Gly Ser Cys
50 55 60
Leu Thr Thr Tyr Gly Tyr Thr Ala Gly Val Tyr Val Met Ile Phe Asp
65 70 75 80
Cys Asn Thr Ala Val Arg Glu Ala Thr Leu Trp Gln Ile Trp Gly Asn
85 90 95

Gly Thr Ile Ile Asn Pro Arg Ser Asn Leu Val Leu Ala Ala Ser Ser
100 105 110

Gly Ile Lys Gly Thr Thr Leu Thr Val Gln Thr Leu Asp Tyr Thr Leu
115 120 125

Gly Gln Gly Trp Leu Ala Gly Asn Asp Thr Ala Pro Arg Glu Val Thr
130 135 140

Ile Tyr Gly Phe Arg Asp Leu Cys Met Glu Ser Asn Gly Gly Ser Val
145 150 155 160

Trp Val Glu Thr Cys Val Ser Ser Gln Lys Asn Gln Arg Trp Ala Leu
165 170 175

Tyr Gly Asp Gly Ser Ile Arg Pro Lys Gln Asn Gln Asp Gln Cys Leu
180 185 190

Thr Cys Gly Arg Asp Ser Val Ser Thr Val Ile Asn Ile Val Ser Cys
195 200 205

Ser Ala Gly Ser Ser Gly Gln Arg Trp Val Phe Thr Asn Glu Gly Ala
210 215 220

Ile Leu Asn Leu Lys Asn Gly Leu Ala Met Asp Val Ala Gln Ala Asn
225 230 235 240

Pro Lys Leu Arg Arg Ile Ile Ile Tyr Pro Ala Thr Gly Lys Pro Asn
245 250 255

Gln Met Trp Leu Pro Val Pro Gly Gly Tyr His
260 265

<210> 5

<211> 72

<212> DNA

<213> Viscum album

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gatgttacat gt 72

<210> 6

<211> 17

<212> PRT

<213> Viscum album

<400> 6

Ser Ser Ser Glu Val Arg Tyr Trp Pro Leu Val Ile Arg Pro Val Ile

1

5

10

15

Ala

<210> 7

<211> 756

<212> DNA

<213> Viscum album

<400> 7

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atcacgcttc tccgagatta tgtctcaagc ggaagctttt ccaatgagat accactcttg 120
cgtcagtcta cgatccccgt ctccgatgcg caaagatttg tcttggtgga gctcaccaac 180
caggggggag actcgatcac ggccgccatc gacgttacca atctgtacgt cgtgggttac 240
caagcaggcg accaatccta ctttttgctg gacgcaccac gcggcgcgga aacgcctctc 300
ttcacgggca ccaccgatc ctctctccca ttcaacggaa gctaccctga tctggagcga 360
tacgccggac atagggacca gatccctctc ggtatagacc aactcattca atccgtcacg 420
gcgcttcggt ttcggggcgg cagcacgcgt acccaagctc gttcgatttt aatcctcatt 480
cagatgatct ccgaggccgc cagattcaat cccatcttat ggagggctcg ccaatacatt 540
aacagtgggg cgtcatttct gccagacgtg tacatgctgg agctggagac gagttggggc 600
caacaatcca cgcaagtcca gcattcaacc gatggcggtt ttaataacct aattcggttg 660
gctatacccc ccggttaactt cgtgacgttg accaatgttc gcgacgtgat cgccagcttg 720
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<210> 8

<211> 252

<212> PRT

<213> Viscum album

<400> 8

Tyr Glu Arg Ile Arg Leu Arg Val Thr His Gln Thr Thr Gly Glu Glu

1

5

10

15

Tyr Phe Arg Phe Ile Thr Leu Leu Arg Asp Tyr Val Ser Ser Gly Ser

20

25

30

Phe Ser Asn Glu Ile Pro Leu Leu Arg Gln Ser Thr Ile Pro Val Ser

35

40

45

Asp Ala Gln Arg Phe Val Leu Val Glu Leu Thr Asn Gln Gly Gly Asp

50

55

60

Ser Ile Thr Ala Ala Ile Asp Val Thr Asn Leu Tyr Val Val Ala Tyr

65 70 75 80

Gln Ala Gly Asp Gln Ser Tyr Phe Leu Arg Asp Ala Pro Arg Gly Ala
85 90 95

Glu Thr His Leu Phe Thr Gly Thr Thr Arg Ser Ser Leu Pro Phe Asn
100 105 110

Gly Ser Tyr Pro Asp Leu Glu Arg Tyr Ala Gly His Arg Asp Gln Ile
115 120 125

Pro Leu Gly Ile Asp Gln Leu Ile Gln Ser Val Thr Ala Leu Arg Phe
130 135 140

Pro Gly Gly Ser Thr Arg Thr Gln Ala Arg Ser Ile Leu Ile Leu Ile
145 150 155 160

Gln Met Ile Ser Glu Ala Ala Arg Phe Asn Pro Ile Leu Trp Arg Ala
165 170 175

Arg Gln Tyr Ile Asn Ser Gly Ala Ser Phe Leu Pro Asp Val Tyr Met
180 185 190

Leu Glu Leu Glu Thr Ser Trp Gly Gln Gln Ser Thr Gln Val Gln His
195 200 205

Ser Thr Asp Gly Val Phe Asn Asn Pro Ile Arg Leu Ala Ile Pro Pro
210 215 220

Gly Asn Phe Val Thr Leu Thr Asn Val Arg Asp Val Ile Ala Ser Leu
225 230 235 240

Ala Ile Met Leu Phe Val Cys Gly Glu Arg Pro Ser
245 250

<210> 9
<211> 789
<212> DNA
<213> Viscum album

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aagtccaaca atgatccgaa tcagttgtgg acgatcaaaa gggatggaac cattcgatcc 180
aatggcagct gcttgaccac gtatggctat actgctggcg tctatgtgat gatcttcgac 240
tgtaatactg ctgtgcggga ggccactctt tggcagatat ggggcaatgg gaccatcatc 300
aatccaagat ccaatctggt tttggcagca tcactctggaa tcaaaggcac tacgcttacg 360

gtgcaaacac tggattacac gttgggacag ggctggcttg ccggtaatga taccgcccc 420
 cgcgaggtga ccataatgg gttcaggac ctttgcattg aatcaaatgg agggagtgtg 480
 tgggtggaga cgtgcgtgag tagccaaaag aaccaaagat gggctttgta cggggatggt 540
 tctatacgcc ccaaacaaaa ccaagaccaa tgcctcacct gtgggagaga ctccgtttca 600
 acagtaatca atatatgttag ctgcagcgtt ggatcgtctg ggcagcgtat ggtgtttacc 660
 aatgaagggg ccattttgaa tttaaagaat gggttgcca tggatgtggc gcaagcaaat 720
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<210> 10

<211> 263

<212> PRT

<213> Viscum album

<400> 10

Asp Asp Val Thr Cys Ser Ala Ser Glu Pro Thr Val Arg Ile Val Gly
 1 5 10 15

Arg Asn Gly Met Cys Val Asp Val Arg Asp Asp Asp Phe Arg Asp Gly
 20 25 30

Asn Gln Ile Gln Leu Trp Pro Ser Lys Ser Asn Asn Asp Pro Asn Gln
 35 40 45

Leu Trp Thr Ile Lys Arg Asp Gly Thr Ile Arg Ser Asn Gly Ser Cys
 50 55 60

Leu Thr Thr Tyr Gly Tyr Thr Ala Gly Val Tyr Val Met Ile Phe Asp
 65 70 75 80

Cys Asn Thr Ala Val Arg Glu Ala Thr Leu Trp Gln Ile Trp Gly Asn
 85 90 95

Gly Thr Ile Ile Asn Pro Arg Ser Asn Leu Val Leu Ala Ala Ser Ser
 100 105 110

Gly Ile Lys Gly Thr Thr Leu Thr Val Gln Thr Leu Asp Tyr Thr Leu
 115 120 125

Gly Gln Gly Trp Leu Ala Gly Asn Asp Thr Ala Pro Arg Glu Val Thr
 130 135 140

Ile Tyr Gly Phe Arg Asp Leu Cys Met Glu Ser Asn Gly Gly Ser Val
 145 150 155 160

Trp Val Glu Thr Cys Val Ser Ser Gln Lys Asn Gln Arg Trp Ala Leu
 165 170 175

Tyr Gly Asp Gly Ser Ile Arg Pro Lys Gln Asn Gln Asp Gln Cys Leu
180 185 190

Thr Cys Gly Arg Asp Ser Val Ser Thr Val Ile Asn Ile Val Ser Cys
195 200 205

Ser Ala Gly Ser Ser Gly Gln Arg Trp Val Phe Thr Asn Glu Gly Ala
210 215 220

Ile Leu Asn Leu Lys Asn Gly Leu Ala Met Asp Val Ala Gln Ala Asn
225 230 235 240

Pro Lys Leu Arg Arg Ile Ile Ile Tyr Pro Ala Thr Gly Lys Pro Asn
245 250 255

Gln Met Trp Leu Pro Val Pro
260

<210> 11

<211> 48

<212> DNA

<213> Viscum album

<400> 11

tcctctgagg tgcgctattg gccgctgggc atacgaccgc tgatagcc

48

<210> 12

<211> 16

<212> PRT

<213> Viscum album

<400> 12

Ser Ser Glu Val Arg Tyr Trp Pro Leu Val Ile Arg Pro Val Ile Ala
1 5 10 15

<210> 13

<211> 94

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Synthetic gene
encoding amino acids 53-78 of human P2 protein

<400> 13

gtaccgggtg gcggtcgtac cgaatccacc ttcaaaaaca ccgaaatctc cttcaaactg 60

ggtcaggaat tcgaagaaac caccgctgac aact

94

<210> 14

<211> 26

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Amino acids
53-78 of human P2 protein

<400> 14

Arg Thr Glu Ser Thr Phe Lys Asn Thr Glu Ile Ser Phe Lys Leu Gly
1 5 10 15

Gln Glu Phe Glu Glu Thr Thr Ala Asp Asn
20 25

<210> 15

<211> 75

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Fig. 20:
Synthetic linker cassette for providing modularity
at the 3' end of rMLB delta lalpha lbeta

<400> 15

caccggtaaa ccgaaccaga tgtggctgcc ggtaccgtag taacgctcct cgtcgaccta 60
gtaaggatcc ctcga 75

<210> 16

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Fig. 20: amino
acid sequence encoded by portion of SEQ ID NO: 15

<400> 16

Thr Gly Lys Pro Asn Gln Met Trp Leu Pro Val Pro
1 5 10

<210> 17

<211> 82
<212> DNA
<213> Artificial S quence

<220>

<223> Description of Artificial Sequence:Fig. 21:
Synthetic linker cassette for providing modularity
at the 3'end of rMLB Delta lalpha lbeta 2gamma
with affinity module ("His-Tag").

<400> 17
ccggtaaacc gaaccagatg tggtgccgg taccgggtgg tggatatcat caccaccatc 60
accactagta actcctcgga tc 82

<210> 18
<211> 21
<212> PRT
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Amino acid
sequence encoded by a portion of SEQ ID NO: 17

<400> 18
Gly Lys Pro Asn Gln Met Trp Leu Pro Val Pro Gly Gly Gly Tyr His
1 5 10 15

His His His His His
20

<210> 19
<211> 26
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Codon exchange
rMLB D23A

<400> 19
catgtgcgtg gccgtccgag atgacg 26

<210> 20
<211> 27
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Fig. 22:
Mutagenic oligonucleotides for inactivating
carbohydrate binding sites in rMLB. - 1alpha2
(W38A). -

<400> 20

cagatacagt tggcgccctc caagtcc

27

<210> 21

<211> 61

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Fig. 22:
Mutagenic oligonucleotides for inactivating
carbohydrate binding sites in rMLB. - 1beta (Y68S,
Y70S, Y75S, F79S). -

<400> 21

gctgcttgac cacgtctggc tctactgctg gcgtctctgt gatgatctcc gactgtaata 60
c 61

<210> 22

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Fig. 22:
Mutagenic oligonucleotides for inactivating
carbohydrate binding sites in rMLB. - 1beta1
(D235A). -

<400> 22

gggttgccca tggtgtggc gcaagc

26

<210> 23

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Fig. 22
Mutagenic oligonucleotides for inactivating
carbohydrat binding sites in rMLB. - 2gamma2
(Y249A). -

<400> 23
cgaataatca tcgctcctgc cacagg 26

<210> 24
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Fig. 22:
Mutagenic oligonucleotides for inactivating
carbohydrate binding sites in rMLB. - pT7 EcoRV to
SspI. -

<400> 24
cttccttttt caatattatt gaagcattta tcagg 35

<210> 25
<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Fig. 22:
Mutagenic oligonucleotides for inactivating
carbohydrate binding sites in rMLB. - pT7 SspI to
EcoRV. -

<400> 25
cttccttttt cgatatcatt gaagcattta tcagg 35

<210> 26
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Fig. 23:
Mutagenic oligonucleotides for constructing
modular ITF gene cassettes. - pT7 Delta NdeI to
StuI. -

<400> 26
ctttaagaag gagatataca ggcctacgag aggctaagac 40

<210> 27
<211> 33

<212> DNA
<213> Artificial S quence

<220>
<223> Description of Artificial Sequence:Fig. 23:
Mutagenic oligonucleotides for constructing
modular ITF gene cassettes. - rMLB silent NheI. -

<400> 27
gttacctgca gtgctagcga acctacggtg cgg

33

<210> 28
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Fig. 23:
Mutagenic oligonucleotides for constructing
modular ITF gene cassettes. - rMLA Delta AgeI. -

<400> 28
cccaccagac caccggcgaa gaatatttcc gg

32

<210> 29
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Fig. 23:
Mutagenic oligonucleotides for constructing
modular ITF gene cassettes.

<400> 29
gtttgtatgc ggagagcgtc cctcgagctc tgaggtgcgc

40

<210> 30
<211> 43
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Fig. 23:
Mutagenic oligonucleotides for constructing
modular ITF gene cassettes. - rMLB Delta EcoNI to
AgeI. -

<400> 30
ccgaataatc atcgctccgg ccaccggtaa accaaatcaa atg

43

<210> 31
<211> 11
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Flanking region
of the ProML gene cassette in expression vector
pT7ProML

<400> 31
tacatatgta c

11

<210> 32
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Flanking region
of the ProML gene cassette in expression vector
pT7ProML

<400> 32
ccatgataag gatcctctag

20

<210> 33
<211> 9
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:Flanking region
of the IML gene cassette in expression vector
PIML-02-P

<400> 33
caggcctac

9

<210> 34
<211> 34
<212> DNA
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Flanking region
of the IML gene cassette in expression vector
PIML-02-P

<400> 34
cactagtaac tcctcgatc ctctagagtc gacc

34

<210> 35

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Modulator
module peptide

<400> 35
Lys Asp Glu Leu
1

<210> 36

<211> 4

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Modulator
module peptide

<400> 36
His Asp Glu Leu
1

<210> 37

<211> 17

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:Portion of the
ML propeptide

<400> 37
Ser Ser Ser Glu Val Arg Tyr Trp Pro Leu Val Ile Arg Pro Val Ile
1 5 10 15

Ala

<210> 38

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: A degradation
product of myelin basic protein.

<400> 38

Val His Phe Phe Lys Asn Ile Val Thr Pro Arg Thr Pro

1

5

10